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SUBSPECIFIC STATUS OF LEAST TERN POPULATIONS IN TEXAS: NORTH AMERICAN IMPLICATIONS

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Abstract.—Analyses of seven bill, leg, wing, and plumage characters measured from 267 museum specimens of adult Least Terns (*Sterna antillarum*) revealed significant morphological differences between sexes but not among three subspecies (*antillarum, athalassos,* and *browni*) currently recognized in North America. While individual morphometric characters sometimes did not overlap between a pair of subspecies, discriminant analysis employing criteria developed from the complete morphometric data set correctly classified >90% of *antillarum* specimens but misclassified 39% and 51% of *athalassos* and *browni* specimens, respectively. Cluster analysis did not segregate specimens into groups consistent with current subspecific taxonomy. Electrophoretic variation in proteins encoded by 50 loci revealed no genetic distinctions between *S. a. antillarum* and *S. a. athalassos* for 22 specimens from four breeding sites on the Texas coast, Rio Grande, and Texas panhandle rivers. These data illustrated continued difficulty in distinguishing endangered and nonendangered "forms" of the same species. Distinctions proposed in original descriptions of North American Least Tern subspecies are not sufficiently definitive; we recommend reassessment of subspecies within the entire species taxon. *Received 20 May 1991, accepted 22 Oct. 1991.*

Three subspecies of Least Terns (*Sterna antillarum,* formerly *S. albifrons*) are recognized from North America (A.O.U. 1957, 1983). Coastal Least Terns (*S. a. antillarum*) nest along the Atlantic seaboard from southern Maine (Hunter 1975) to Florida, the Gulf Coast, and Caribbean islands (A.O.U. 1957, 1983). Interior Least Terns (*S. a. athalassos*) breed in the Mississippi Valley; along tributaries of the Missouri, Arkansas, and Red rivers from North Dakota to Texas; and at scattered sites in the Rio Grande drainage of New Mexico and Texas (Hardy 1957, Downing 1980, Whitman 1988). California Least Terns (*S. a. browni*) are restricted to a breeding range along the Pacific Coast from San Francisco Bay, California, to Baja California, Mexico (California Least Tern Recovery Team 1980).

Number and size of *S. antillarum* breeding populations have declined in recent times owing to disturbance or destruction of nesting and feeding habitat by shoreline development, dredging, diversion and impoundment

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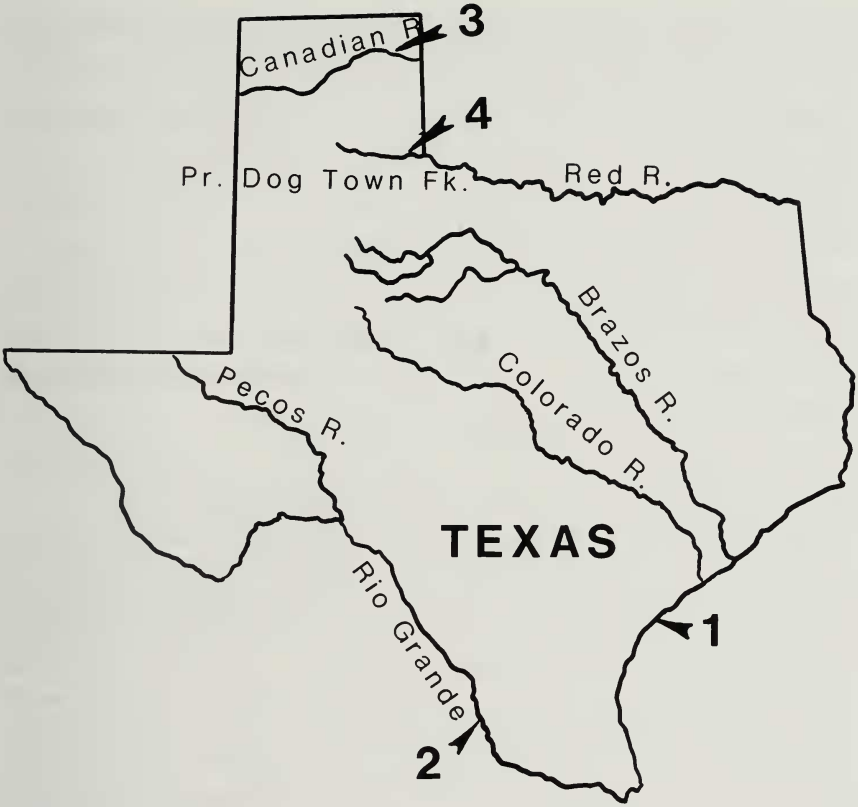

FIG. 1. Collecting localities for samples of Least Terns (*Sterna antillarum*) in (1) Aransas, (2) Zapata, (3) Hemphill, and (4) Childress counties, Texas. Samples from locality (1) represent Coastal Least Terns (*S. a. antillarum*); the remaining samples are referable to Interior Least Terns (*S. a. athalassos*).

of rivers, and recreational activities (e.g., Downing 1973, 1980; California Least Tern Recovery Team 1980; Ducey 1981; Gochfeld 1983). Endangered classification was implemented in 1970 for *S. a. browni* (U.S. Fish and Wildl. Serv. 1970) and in 1985 for *S. a. athalassos* (U.S. Fish and Wildl. Serv. 1985). Surveys during the mid-1980s estimated fewer than 2000 *S. a. athalassos* in breeding populations throughout the Mississippi River and Rio Grande drainages (summarized in Whitman 1988).

Texas is unique in supporting substantial breeding populations of two subspecies of Least Terns. Coastal Least Terns, although nesting in areas subject to disturbance by humans, are relatively abundant along the Gulf Coast, while Interior Least Terns occur in limited numbers along the Red and Canadian rivers (Fig. 1) of the Texas Panhandle (Downing 1980,

Texas Colonial Waterbird Society 1982). Least Terns also nest along the lower Rio Grande (Fig. 1) at Amistad (Val Verde Co.) and Falcon (Zapata Co.) reservoirs and near Laredo (Webb Co.), Texas. These latter birds are considered to represent *S. a. athalassos* by virtue of riverine nesting habitat and geographic inclusion in endangered classification (U.S. Fish and Wildl. Serv. 1985) but exist in closer proximity to Coastal Least Tern populations than to other populations of Interior Least Terns. Accurate taxonomic assignment of lower Rio Grande populations is important to assessments of population levels for the endangered Interior Least Tern (e.g., Downing 1980, Ducey 1981, Texas Colonial Waterbird Society 1982, Whitman 1988). Ability to distinguish subspecies through robust taxonomic criteria is important to developing conservation strategies for endangered taxa (Avise and Nelson 1989). Development of conservation and enforcement strategies related to differential classification of *antillarum* and *athalassos* under state and federal laws exemplified a need to apply more modern techniques in evaluating taxonomic status.

Difficulties in discriminating among subspecies of *S. antillarum* were exacerbated by absence of quantitative analyses of bill, wing, and leg measures in original subspecies descriptions. Indeed, suggested diagnostic characters for *S. a. browni* (Mearns 1916) and *S. a. athalassos* (Burleigh and Lowery 1942) comprised subtle differences in coloration of plumage (upperparts and nape) and bills. Specifically, Mearns (1916) described *S. a. browni,* in comparison with *S. a. antillarum,* as (1) smaller (not definitively quantified), (2) black of crown more prolonged backward, (3) usually three black outer primaries, (4) dorsum darker gray and venter more grayish, and (5) bill more narrowly tipped with black, often without any black. Burleigh and Lowery (1942) disputed the distinction of *S. a. browni* from *S. a. antillarum* and reported that *S. a. athalassos* differed from *S. a. antillarum* only in having "much darker coloration of the upper parts" and having "little or no distinction between color of the back and that of the hind neck." Number of dark primary feathers, considered by Mearns (1916) as a good character with which to distinguish *S. a. browni* (usually three) from *S. a. antillarum* (two), later was reported as highly variable among individuals of each subspecies (Burleigh and Lowery 1942). Furthermore, courtship, nesting behavior, and vocalizations are similar in Least Terns from Atlantic and Pacific coasts (Massey 1976), and behavior and habitat use characteristics are similar for coastal and interior populations (Hardy 1957, Downing 1980, Boyd and Thompson 1985, Whitman 1988). Subspecific taxonomy of Least Terns hinged on separate breeding ranges, but such a distinction was vague in the case of the Rio Grande population and meaningless for terns in migration.

We examined patterns of variation in eight bill, leg, wing, and plumage

characters among specimens of *S. a. antillarum, S. a. athalassos,* and *S. a. browni* to test hypothesized differences among subspecies and if validated, to develop criteria with which to distinguish the subspecies under field conditions. In addition, we surveyed electrophoretic variation in proteins among samples of *S. a. antillarum* and *S. a. athalassos* from nest sites along the Texas Gulf Coast, lower Rio Grande, and in the Texas Panhandle. Analyses were designed to test for discrete biochemical markers which might serve to distinguish subspecies, permit taxonomic assignment of samples from nest sites on the lower Rio Grande, and evaluate genetic consequences of small population size (decreased genetic variability and increased genetic differentiation) that may have accompanied the decline of Interior Least Terns.

MATERIALS AND METHODS

Morphological variation.—Museum specimens of *S. a. antillarum, S. a. athalassos,* and *S. a. browni* were obtained from the American Museum of Natural History, Cleveland Museum of Natural History, Field Museum of Natural History, Florida State Museum, Kansas State Museum of Natural History, U.S. National Museum of Natural History, and Texas Cooperative Wildlife Collection at Texas A&M Univ. We examined adult (breeding plumage only) specimens taken from the United States and adjacent Caribbean fringe of Mexico, Cuba, and the West Indies within the described range of the several subspecies (A.O.U. 1957, 1983). Geographic distribution was represented similarly among male and female specimens for each subspecies. Specifically, 83% of 192 *S. a. antillarum* specimens represented Gulf and Atlantic coastal sites from Texas to Massachusetts; the other 17% were collected from scattered sites in the West Indies and Gulf Coast of Mexico/Central America. All 36 *S. a. athalassos* specimens were from sites at least 250 km interior from coastal areas and represented states from Texas and Louisiana to Kansas in the Mississippi River drainage. The 39 *S. a. browni* specimens were 90% from California and 10% from the west coast of Mexico.

Date, location of collection, sex, and subspecific designation were recorded from specimen tags. Measurement of external characteristics followed Baldwin et al. (1931) and included: CULMEN—length of exposed culmen (from point at which feathers cease to hide the culmen to the culmen tip); MANDIBLE—length of lower mandible from gonys to mandible tip; TARSOTAR—diagonal of tarsus length (distance between tibiotarsal-tarsometatarsal joint and last undivided scute); MIDTOE—length of middle toe minus claw; and WINGCHOR—chord of closed folded flattened wing. Wings were measured with a millimeter ruler; all other external characters were measured with vernier calipers (accurate to 0.1 mm). The number of dark distal primaries on each wing (PRIMARYR and PRIMARYL) and presence of bill tip coloration were noted because these were important characters in original subspecies descriptions.

Relative reflectance of the mid-region of the back of each specimen was quantified by comparison with a Kodak Reflection Density Guide. This standard guide was graduated in 24 steps (or patches) that varied from white (0.0) to black (2.0). Each specimen was viewed independently by four observers under the same lighting conditions and scored by determining closest match between scale patch and specimen back reflectance (or coloration). Four scores for each specimen were averaged to produce a composite reflectance value (DENSITY).

We subjected complete records for 267 adult specimens to factorial analysis of variance (ANOVA), discriminant analysis, and cluster analysis (SAS Inst., Inc. 1982). Univariate summary statistics were calculated, and two-factor ANOVA was performed for all morphometric variables to examine individual character traits between sexes and among presumed subspecies. Scheffe's test (Zar 1974:159) was used for means separation. Normality of variables was verified with the *D* statistic of PROC UNIVARIATE (SAS Inst., Inc. 1982). Only dark primary counts and plumage reflectance values deviated significantly from normality, but transformation did not change variance pattern, so analyses were performed with original variables to preserve the scale. Stepwise discriminant analysis was used to select morphometric characters that were most efficient in separating sex or population categories (characters selected/retained at $P \leq 0.15$). Linear discrimination was used to develop criteria from the 267 specimens for distinguishing among the three subspecies currently recognized in North America. A fast cluster procedure (SAS Inst., Inc. 1982) was used to examine patterns in multivariate specimen groupings relative to location of specimen collection. This procedure evaluated the hypothesis that specimens of the three recognized subspecies would segregate into three clusters. At all stages of analysis, variables were evaluated to verify pooling of covariance matrices and pooling or separation of sexes.

Biochemical genetics.—Samples of *S. antillarum* (N = 22) were collected for biochemical genetic analyses from four nesting localities in Texas during the 1985 breeding season (Fig. 1). Coastal Least Terns (*S. a. antillarum*; N = 11) were collected alive from the shore of Aransas Bay near Rockport (Aransas Co.; 28°02′N, 97°02′W). Interior Least Terns (*S. a. athalassos*) were collected alive from sandbar nest sites on the Rio Grande (Zapata Co.; 28°36′N, 99°10′W; N = 7), the Canadian River (Hemphill Co.; 35°53′N, 100°15′W; N = 2), and the Prairie Dog Town Fork of the Red River (Childress Co.; 34°34′N, 100°12′W; N = 2). With the exception of one well-feathered individual included in the Rio Grande sample (a salvage specimen found with acute hydrocephalus), specimens were prefledging chicks taken from widely separated nests within colonies. All samples of Interior Least Terns were collected under a special endangered species subpermit from the U.S. Fish and Wildlife Service regional office in Albuquerque, New Mexico.

Liver, brain, and pectoral muscle extracted from each individual immediately after sacrifice in the lab were prepared and subjected to vertical starch-gel electrophoresis according to protocols of Siciliano and Shaw (1976) and Morizot et al. (1977). Histochemical stains were prepared as described in Siciliano and Shaw (1976) and Harris and Hopkinson (1976). Products of 50 protein loci were resolved in the electrophoretic analysis (Table 1). For multilocus protein systems, loci were designated numerically in order of decreasing anodal electrophoretic mobility of isozymes. Alleles at polymorphic loci were designated alphabetically in order of decreasing anodal electrophoretic mobility of protein products.

Genic data were analyzed as single-individual genotypes with BIOSYS-1 (Swofford and Selander 1981). Heterozygosity at individual loci (h), average individual heterozygosity over all loci ($\bar{H}$), and percentage of loci polymorphic (P; 0.95 frequency criterion) were determined by direct count. Genotypic frequencies within populations were tested for conformance to Hardy-Weinberg proportions (chi-square goodness of fit; Sokal and Rohlf 1969). Chi-square contingency-table analyses tested heterogeneity of allelic frequencies among populations (Workman and Niswander 1970).

Fixation indices (F_{ST}; Wright 1978) were calculated according to Nei and Chesser (1983) as modified by Van Den Bussche et al. (1986) to gauge the amount of genetic variance attributable to differences among collecting localities. Coefficients of genetic similarity (S; Rogers 1972), genetic distance (D; Nei 1972), and unbiased genetic distance (D_u; Nei 1978) were calculated for all pairwise comparisons of populations. Phenetic relationships among populations were depicted in a dendrogram (unweighted pair-group method using arithmetic

averages; Sneath and Sokal 1973) generated from the matrix of coefficients of *D.* Standard errors of branching points in the dendrogram were calculated according to methods of Nei et al. (1985).

RESULTS

Morphometric analyses.—Univariate statistics for 267 specimens separated by sex revealed substantial overlap of *athalassos* and *browni* with *antillarum* for the eight morphological measures (Table 2). Notable deviations from this overlap were (1) minimum CULMEN measures for both sexes of *athalassos* and *browni* which were 1.2% to 5% smaller than for smallest *antillarum* of respective sexes, and (2) only one specimen, a female *athalassos,* had five dark primaries. Evaluation of bill tip coloration indicated that darkening of the bill tip was present in >75% of specimens among all subspecies and fading or patchiness of the dark coloration defied standard measurement, thus that character was not analyzed further.

Factorial ANOVA revealed clear distinctions between sexes within subspecies for all variables except MIDTOE, TARSOTAR, and WINGCHOR (F range = 11.79 to 66.83, df = 1, 261, $P < 0.001$). Therefore, sexes were separated for subsequent analyses. Despite extensive overlap, factorial ANOVA indicated that *antillarum* had longer CULMEN (F = 24.94, df = 2, 261, $P < 0.001$), *browni* had longer WINGCHOR (F = 8.01, df = 2, 261, $P < 0.001$), and DENSITY differed among all subspecies (F = 35.90, df = 2, 261, $P < 0.001$). None of these variables extensively distinguished individual specimens to a single subspecies. PRIMARYL and PRIMARYR were highly correlated; therefore PRIMARYL was excluded from further analyses. Discriminant analysis correctly classified sex for >85% of males in all subspecies and for female *browni* but classified only 43% and 58% correctly for females of *athalassos* and *antillarum,* respectively. Discrimination based on CULMEN, DENSITY, and MANDIBLE (characters selected from stepwise procedures) were 92.1%, 93.5%, and 98.0% as accurate in distinguishing sex of *browni, athalassos,* and *antillarum,* respectively, as were criteria based on all variables.

Linear discrimination criteria correctly classified subspecies for 90.4% and 94%, respectively, of male and female specimens originally assigned to *antillarum,* but classified only 41% to 67% correct for *athalassos* and *browni* specimens of both sexes. Relatively high percentages of correct *antillarum* classifications and >30% of both sexes of *athalassos* and *browni* misassigned to *antillarum* indicated influence of *antillarum* characters among all presumed subspecies. Cluster analysis did not reveal segregation into three distinct groupings among specimens of three presumed subspecies (Table 3). Most specimens grouped in one to three clusters that contained a broad mix of the three subspecies. This analysis is not sensitive

TABLE 1

PROCEDURES USED TO RESOLVE ELECTROPHORETIC VARIATION IN SAMPLES OF COASTAL AND INTERIOR LEAST TERNS FROM TEXAS

Locus	E.C. No.	Symbol[a]	Tissue[b]	Buffer[c]
Aconitase 2 (mitochondrial)	4.2.1.3	CO2	M	TEB
Adenosine deaminase	3.5.4.4	ADA	M	TEB
Alcohol dehydrogenase	1.1.1.1	ADH	L	TC
Carbonic anhydrase 1	4.2.1.1	CA1	M	TEB
Carbonic anhydrase 2	4.2.1.1	CA2	M	TEB
Creatine kinase 1	2.7.3.2	CK1	B	TC
Creatine kinase 2	2.7.3.2	CK2	B	TC
Enolase	4.2.1.11	ENO	B	TC
Esterase 1 (α-naphthyl esters)	3.1.1.1	ES1	M	TEB
Esterase 2 (α-naphthyl esters)[d]	3.1.1.1	ES2	M	TEB/TC
Esterase 3 (methylumbelliferyl acetate)	3.1.1.1	ES3	M	TEB
Esterase 4 (methylumbelliferyl acetate)	3.1.1.1	ES4	M	TEB
Fumarate hydratase	4.2.1.2	FH	M	TEB
Glucose phosphate isomerase	5.3.1.9	GPI	M	TC
α-glucosidase	3.2.1.20	GAA	L	TC
Glutamate dehydrogenase	1.4.1.3	GLUD	L	TEB
Glutamic-oxaloacetic transaminase 1	2.6.1.1	GOT1	L	TC
Glutamic-pyruvate transaminase 1	2.6.1.2	GPT1	M	TEB
Glutamic-pyruvate transaminase 2	2.6.1.2	GPT2	M	TEB
Glutathione reductase 1	1.6.4.2	GSR1	L	TEB
Glutathione reductase 2	1.6.4.2	GSR2	L	TEB
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	GAPD	M	TC
Glycerate dehydrogenase	1.1.1.29	GLYDH	L	TC
Glycerol-3-phosphate dehydrogenase	1.1.1.8	GPD	L	TC
Glyoxalase 1	4.4.1.5	GLO	M	TEB
Guanine deaminase	3.5.4.3	GDA	L	TEB
Guanylate kinase	2.7.4.8	GUK	B	TEB

TABLE 1
CONTINUED

Locus	E.C. No.	Symbol[a]	Tissue[b]	Buffer[c]
Inosine triphosphatase	3.1.6.3	ITP	M	TC
Isocitrate dehydrogenase 1	1.1.1.42	IDH1	L	TEB
Isocitrate dehydrogenase 2	1.1.1.42	IDH2	L	TEB
Lactate dehydrogenase 1	1.1.1.27	LDH1	B	TC
Lactate dehydrogenase 2	1.1.1.27	LDH2	B	TC
Malate dehydrogenase 1	1.1.1.37	MDH1	M	TC
Malate dehydrogenase 2	1.1.1.37	MDH2	M	TC
Mannose phosphate isomerase	5.3.1.8	MPI	M	TEB
α-mannosidase	3.2.1.24	MAN	M	TEB
Muscle protein[e]		MP	M	TC
Nucleoside phosphorylase	3.4.2.1	NP	B	TEB
Peptidase 1 (glycyl-leucine)	3.4.11	PEP1	M	TEB
Peptidase 2 (leucyl-glycyl-glycine)	3.4.11	PEP2	M	TEB
Peptidase 3 (leucyl-glycyl-glycine)	3.4.11	PEP3	L	TEB
Peptidase 4 (leucyl-glycyl-glycine)	3.4.11	PEP4	L	TEB
Phosphoglucomutase 1	2.7.5.1	PGM1	M	TEB
Phosphogluconate dehydrogenase	1.1.1.44	PGD	M	TC
Phosphoglyceratemutase	2.7.5.3	PGAM	M	TEB
Pyruvate kinase	2.7.1.40	PK	B	TC
Superoxide dismutase 1	1.15.1.1	SOD1	L	TEB
Superoxide dismutase 2	1.15.1.1	SOD2	M	TEB
Triosephosphate isomerase	5.3.1.1	TPI	B	TEB
Uridine monophosphate kinase 1	2.7.4	UMPK1	B	TC

[a] Locus symbols follow standardized nomenclature for mapped human genes (McAlpine et al. 1987).
[b] B = brain; L = liver; M = pectoral muscle.
[c] TC = tris-citrate, pH 7.0; TEB = tris-EDTA-borate, pH 8.0 (Siciliano and Shaw 1976).
[d] Proteins encoded by $ES2^A$ and $ES2^B$ comigrated on TEB buffer but separated on TC buffer.
[e] Five zones of general protein activity were apparent from muscle on TC gels; nevertheless, because no allelic variation was observed, MP is reported as a single locus.

TABLE 2

UNIVARIATE STATISTICS FOR EIGHT PHYSICAL MEASURES FROM 267 LEAST TERN MUSEUM SPECIMENS REPRESENTING THREE PRESUMED SUBSPECIES IN NORTH AMERICA

Subspecies	Sex	Variable	$\bar{x}$	SE[a]	Minimum	Maximum	CV (%)[b]
antillarum	M	Culmen	28.2	0.13	24.4	31.3	5.1
	(N = 125)	Mandible	17.1	0.14	13.7	21.5	9.1
		Tarsotar	15.5	0.04	14.4	17.0	3.1
		Midtoe	13.2	0.05	11.8	14.4	4.1
		Wingchor	166.9	0.47	153.0	180.0	3.2
		PrimaryL	2.1	0.04	1.0	4.0	24.2
		PrimaryR	2.1	0.05	1.0	4.0	24.7
		Density	0.5	0.01	0.4	0.7	14.2
athalassos	M	Culmen	26.6	0.20	23.1	28.3	4.1
	(N = 29)	Mandible	16.3	0.19	14.1	18.2	6.4
		Tarsotar	15.3	0.08	14.5	16.1	3.0
		Midtoe	13.3	0.08	12.3	14.2	3.1
		Wingchor	167.8	0.82	157.0	177.0	2.6
		PrimaryL	2.3	0.09	2.0	3.0	20.6
		PrimaryR	2.3	0.09	2.0	3.0	20.4
		Density	0.6	0.01	0.5	0.7	7.5
browni	M	Culmen	26.8	0.25	23.8	29.3	4.9
	(N = 27)	Mandible	16.4	0.23	13.6	18.4	7.5
		Tarsotar	15.5	0.10	14.1	16.3	3.4
		Midtoe	13.2	0.08	12.2	13.9	3.1
		Wingchor	170.2	0.71	162.0	177.0	2.2
		PrimaryL	2.6	0.10	2.0	3.0	19.3
		PrimaryR	2.6	0.10	2.0	3.0	19.8
		Density	0.5	0.01	0.5	0.6	9.2

TABLE 2
CONTINUED

Subspecies	Sex	Variable	$\bar{x}$	SE[a]	Minimum	Maximum	CV (%)[b]
antillarum	F	Culmen	26.7	0.15	24.2	30.0	4.6
	(N = 67)	Mandible	16.3	0.16	13.1	20.1	8.0
		Tarsotar	15.6	0.06	14.6	16.5	2.9
		Midtoe	13.2	0.06	11.8	14.4	3.8
		Wingchor	166.4	0.52	154.0	173.0	2.5
		PrimaryL	2.3	0.07	1.0	4.0	24.1
		PrimaryR	2.3	0.07	1.0	4.0	23.8
		Density	0.6	0.01	0.4	0.7	11.6
athalassos	F	Culmen	25.8	0.50	23.9	27.6	5.1
	(N = 7)	Mandible	15.3	0.35	14.3	16.7	6.0
		Tarsotar	15.3	0.10	15.0	15.8	1.7
		Midtoe	13.2	0.24	12.2	14.0	4.9
		Wingchor	166.3	1.60	159.0	172.0	2.5
		PrimaryL	3.0	0.38	2.0	5.0	33.3
		PrimaryR	3.0	0.38	2.0	5.0	33.3
		Density	0.6	0.02	0.6	0.7	7.8
browni	F	Culmen	25.2	0.40	23.0	26.8	5.4
	(N = 12)	Mandible	15.8	0.35	13.4	17.9	7.7
		Tarsotar	15.4	0.08	15.0	15.9	1.8
		Midtoe	12.7	0.13	11.7	13.3	3.5
		Wingchor	169.6	1.22	161.0	174.0	2.5
		PrimaryL	2.7	0.14	2.0	3.0	18.5
		PrimaryR	2.6	0.15	2.0	3.0	19.9
		Density	0.6	0.01	0.6	0.7	6.3

[a] SE = standard error defined as standard deviation of the sample mean.
[b] CV = coefficient of variation; the sample standard deviation divided by the sample mean.

TABLE 3

MULTIVARIATE CLUSTER PATTERNS FOR 267 NORTH AMERICAN LEAST TERN SPECIMENS

Sex	N	Max. clusters[a]	Cluster	Percent of subspecies in cluster[b]		
				antillarum	*athalassos*	*browni*
Male	181	3	1	15.2	20.7	29.6
			2	66.4	69.0	66.7
			3	18.4	10.3	3.7
Female	86	3	1	10.5	14.3	8.3
			2	29.9	28.6	66.7
			3	59.7	57.1	25.0

[a] See text for rationale of selection for three clusters.
[b] FAST CLUSTER (SAS Inst., Inc. 1982) procedure applied to seven morphometric characters allowing for three clusters.

to detecting how many clusters may effectively categorize the tern specimens, only that the three clusters hypothesized from current subspecies descriptions were not verified.

Biochemical analyses.—Allelic variation was detected at 12 of 50 loci examined (Table 4). In general, allelic frequencies were similar among Texas populations and between "coastal" and "interior" subspecies. At least one allele was shared by each of the four populations at each polymorphic locus. Two alleles were common to all populations at ADA, and three alleles (C, D, and E) were common to all populations at NP. Greatest interpopulational variance in observed allele frequencies occurred at UMPK1. UMPK1^{B} predominated in Coastal Least Terns and the Rio Grande sample of Interior Least Terns, whereas UMPK1^{A} predominated among Interior Least Terns from Canadian and Red rivers (Table 4).

Genotypic frequencies conformed to expectations of Hardy-Weinberg equilibrium at all loci in each population sampled. Furthermore, when all individuals were grouped into a single population, genotypic frequencies deviated significantly from equilibrium proportions only at PGD ($\chi^2 = 3.942$; df = 1; $P = 0.047$). Chi-square contingency table analyses revealed significant heterogeneity in allele frequencies among the four populations of Least Terns at SOD2 ($\chi^2 = 10.23$, df = 3, $P = 0.017$) and UMPK1 ($\chi^2 = 9.48$, df = 3, $P = 0.024$). Allele frequencies were not significantly heterogeneous between the two populations of interior Least Terns from the Texas Panhandle (Canadian and Red rivers). Thus, individuals from these sites were combined into a single population (N = 4) for remaining analyses. Significantly heterogeneous allele frequencies were observed only at UMPK1 between this Panhandle population and coastal ($\chi^2 = 4.22$, df = 1, $P = 0.040$) and Rio Grande populations ($\chi^2 = 8.96$, df = 1, $P = 0.003$).

TABLE 4

ALLELE FREQUENCIES AT POLYMORPHIC LOCI AMONG FOUR TEXAS LEAST TERN POPULATIONS

			Interior		
Locus[a]	Allele	Coastal (N = 11)	Rio Grande (N = 7)	Canadian River (N = 2)	Red River (N = 2)
ADA	A	0.182	0.071	0.500	0.250
	B	0.818	0.929	0.500	0.750
ES1	A		0.071		
	B	1.000	0.929	1.000	1.000
ES2	A	0.864	0.786	1.000	0.750
	B		0.071		
	C	0.136	0.143		0.250
ES3	A		0.071		0.250
	B	1.000	0.929	1.000	0.750
MPI	A	0.045			
	B	0.955	1.000	1.000	1.000
NP	A	0.091			0.250
	B	0.045			
	C	0.273	0.286	0.250	0.250
	D	0.455	0.500	0.500	0.250
	E	0.136	0.214	0.250	0.250
PEP2	A	0.955	1.000	1.000	1.000
	B	0.045			
PEP3	B	1.000	0.929	1.000	1.000
	C		0.071		
PGD	A	0.150	0.200		0.500
	B	0.850	0.800	1.000	0.500
PGM1	A	0.955	1.000	1.000	1.000
	B	0.045			
SOD2	A	1.000	1.000	1.000	0.750
	B				0.250
UMPK1	A	0.450	0.214	0.750	1.000
	B	0.550	0.786	0.250	

[a] Loci are as designated in Table 1.

Average individual heterozygosity ($\bar{H}$) and percentage of loci polymorphic (P) were similar for each population (Table 5); values of these statistics over all individuals, considered as a single population, were $\bar{H} = 0.049$ and $P = 0.100$, respectively. High levels of heterozygosity (h) at the NP locus contributed much of the overall genetic variability in each population (Table 5). Fixation indices (F_{ST}) revealed modest levels of interpopulational differentiation at each polymorphic locus except UMPK1

TABLE 5

HETEROZYGOSITY AT THE POLYMORPHIC LOCI, AND FIXATION INDICES FOR TEXAS LEAST TERNS

Locus or statistic	Heterozygosity (h)[a]			F_{ST}
		Interior		
	Coastal (N = 11)	Rio Grande (N = 7)	Panhandle (N = 4)	
ADA	0.364	0.143	0.750	0.091
ES1	—	0.143	—	0.045
ES2	0.273	0.429	0.250	0.009
ES3	—	0.143	0.250	0.040
MPI	0.091	—	—	0.028
NP	1.000	0.714	1.000	0.012
PEP2	0.091	—	—	0.028
PEP3	—	0.143	—	0.045
PGD	0.100	0.400	—	0.028
PGM1	0.091	—	—	0.028
SOD2	—	—	0.250	0.081
UMPK1	0.300	0.429	0.250	0.279
$\bar{H}$[b]	0.046	0.051	0.055	
P[b]	0.100	0.160	0.140	
F_{ST}[b]				0.081

[a] h = Polymorphic loci heterozygosity.

[b] Average individual heterozygosity over all loci (H), percentage of loci polymorphic (P; 0.95 frequency criterion), and fixation indices (F_{ST}; Nei and Chesser 1983; Van Den Bussche et al. 1986).

(Table 5). Mean fixation index ($\bar{F}_{ST}$) indicated that variance in allele frequencies between populations accounted for only 8.1% of total genetic variance.

Coefficients of genetic similarity (S = 0.974), genetic distance (D = 0.007), and unbiased genetic distance (D_u = 0.003) among Texas Least Terns were consistent with overall genetic uniformity of populations. The estimate of D_u between Coastal Least Terns and the Rio Grande population of Interior Least Terns was 0.00, and the standard error for this value of D_u was undefined. Therefore, although Nei's (1978) coefficients of unbiased genetic distance were deemed more appropriate for comparisons among samples comprising few individuals, coefficients of genetic distance (D; Nei 1972) were used to assess phenetic relationships among the populations of Least Terns examined in this study. In the dendrogram derived from the matrix of D coefficients (Fig. 2), Coastal Least Terns and Interior Least Terns from the Rio Grande were clustered more closely than either of these populations was to the sample of Interior Least Terns from the Texas Panhandle. However, relatively large standard errors for

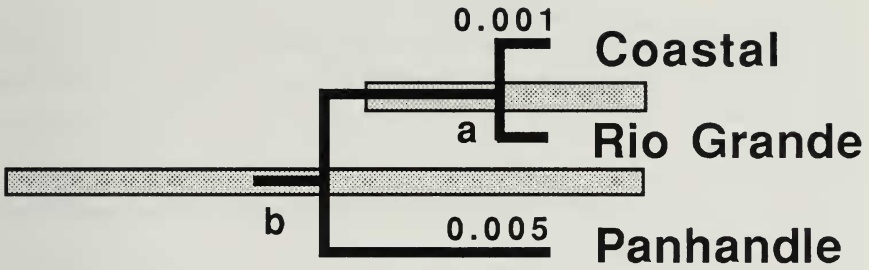

FIG. 2. Dendrogram derived from the matrix of genetic distance coefficients (*D*; Nei 1972). The "Panhandle" population represents Interior Least Terns collected from the Red and Canadian rivers in Texas (localities 3 and 4; Fig. 1). Genetic distances are expressed as the sum of branch lengths separating populations (lengths of terminal branches are shown). Shaded regions represent standard errors of branching points (calculated according to Nei et al. 1985). Standard errors for branching points denoted a and b are ± 0.003 and ±0.007, respectively.

branching points in the dendrogram indicated branch lengths separating the three populations were not different.

DISCUSSION

Bill, wing, leg, toe, and dorsum color variables used for these analyses were the same as or very similar to those reported in previous subspecies descriptions of *S. antillarum.* Individual variables showed mixed consistency with previous qualitative assessments of character differences (i.e., dorsum color but not bill tip or dark primaries in *browni,* Mearns 1916). However, multivariate discrimination did not substantiate distinction of *antillarum* from *athalassos* and *browni* as originally described by Burleigh and Lowery (1942) and Mearns (1916), respectively.

Morphometric analyses presented here seriously question existence of distinctive differences previously proposed in describing *antillarum, athalassos,* and *browni* subspecies. These analyses indicated that *antillarum* largely encompasses the range of measures inherent in North American Least Tern populations for variables examined. This similarity is consistent with Massey's (1976) suggestion that *browni* is indistinguishable behaviorally, vocally, and morphometrically from *antillarum* and Boyd and Thompson's (1985) suggestion that mixing of *antillarum* and *athalassos* may be more common than previously thought. Also, other suggested distinctions between Least Tern subspecies such as nearly mutually exclusive culmen and wing measures described by van Rossem and Hachisuka (1937) for *S. a. mexicanus* and Brodkorb (1940) for *S. a. staebleri* in Mexico relative to other subspecies including *antillarum* were not verified by measurements we report herein.

Estimated average individual heterozygosity among 22 Least Terns from Texas ($\bar{H} = 0.049$) was similar to average heterozygosity calculated by Evans (1987) from reports of biochemical genetic variation in 86 avian taxa ($\bar{H} = 0.044$). However, our estimate exceeded values estimated for Forster's Terns (*Sterna forsteri*) ($\bar{H} = 0.038$) by Hackett (1989) and for populations of California Gulls (*Larus californicus*) by Zink and Winkler (1983: $\bar{H} = 0.028$) and Karl et al. (1987: a range of $\bar{H}$ from 0.027 to 0.035). Our estimates of genetic variability in Least Terns likely were influenced by the large number of loci examined (50; Table 1) and the concomitantly large number of loci displaying allelic variation (12; Table 4). Nevertheless, our analyses did not reveal differences in levels of genetic variability among populations of Least Terns or between coastal and interior subspecies. Similarity in estimates of $\bar{H}$ and P among the three populations examined in this study suggests that genetic variability may not be declining in Interior Least Terns as a result of disruption of nesting habitat.

The only evidence for biochemical genetic differentiation among Least Tern populations from Texas involved variance in UMPK1 allele frequencies between the Panhandle samples and the remaining populations (Table 4). This variance accounted for clustering patterns in the dendrogram of genetic distance (Fig. 2) and the unusually large value of F_{ST} for this locus (0.279, Table 5). Because of small sample sizes, estimated allele frequencies at this locus, as well as others, are subject to large standard errors (± 0.106, ± 0.110, ± 0.117 for coastal, Rio Grande, and Panhandle samples, respectively). Thus, although fixation indices were estimated according to methods intended for small sample sizes (Nei and Chesser 1983), the magnitude of genetic differentiation apparent in UMPK1 allele frequencies may be illusory. We recognize that the use of more sensitive genetic techniques (e.g., analyses of restriction fragment length polymorphisms in mitochondrial DNA; Avise et al. 1987, Avise and Nelson 1989, Zink et al. 1991) or inclusion of samples of Least Terns from a larger portion of the species' range in biochemical genetic analyses (especially from the range of *S. a. browni*) might have revealed patterns of genetic differentiation that correspond with ranges of recognized subspecies. Nonetheless, observed patterns of biochemical genetic differentiation are not consistent with recognition of separate subspecies from interior (Panhandle and Rio Grande) and coastal Texas. These populations are as widely separated (>750 km) as are many of the historic breeding sites for interior and coastal populations in the Mississippi River drainage (Whitman 1988).

Lack of clearly measurable distinctions among the three subspecies, of which two are classified as endangered, complicates appropriate manage-

ment away from breeding grounds. Better understanding of migration routes, wintering areas, and extent of population mixing is necessary to determine how conservation actions directed at breeding grounds should integrate with biology and resources of these populations during the >six months they are outside the United States.

Proper conservation of endangered populations requires more information about interior populations. Additional data are needed to determine whether young fledged at "interior" colonies return there to breed; whether interior birds winter with coastal populations; and, if so, whether coastal birds are recruited into interior breeding flocks. Long-term study of young terns identifiable as to natal area is necessary to ascertain flock movements and use areas for sensitive populations. These considerations are especially warranted for isolated populations throughout the Mississippi River drainage, in the Texas Panhandle, and for the Pecos River drainage in New Mexico where only one Least Tern breeding site is known (Whitman 1988). Also, more consideration should be given to developing management strategies throughout the species range rather than just emphasizing breeding areas of uncertain subspecies.

We recommend that subspecific taxonomy of the *S. antillarum* complex be reassessed because of lack of evidence that populations are morphologically or genetically differentiated and the likelihood that populations are mixing on wintering grounds or migration routes. The subspecies concept is complex but there are compelling arguments that such categorization should at least meet minimum criteria of diagnostic morphological characters, definite geographic restriction, and ecological specialization discussed by Mayr (1970:210–213) or perhaps more importantly, reflect scientific predictiveness (Barrowclough 1982). McKitrick and Zink's (1988) review of the complexity of meaningful subspecies designations in context with current views of avian speciation is pertinent in this instance, as are the views of various authors (see Commentary on Avian Subspecies, Auk 99:593–615, 1982). Research reported here and by Massey (1976) indicated no practical means for distinction among the North American "subspecies" of Least Terns. Discrepancies in other previously suggested mensural distinctions among Least Tern subspecies further cloud the taxonomy and provide little if any predictiveness.

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